The glucose oxidase of honey
II. Stereochemical substrate specificity

In the first paper of this series[1] it was noted that the glucose oxidase of honey had a high substrate specificity, utilizing only glucose. These studies have now been extended to show a preference for β-D-glucose over α-D-glucose, thus establishing the honey enzyme as a β-D-glucose:O_2 oxidoreductase (EC 1.1.3.4). This is the subject of the present report.

α-D-Glucose was obtained from J. T. Baker Chemical Company*, β-D-glucose and catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) from Sigma Chemical Company. The glucose oxidase was prepared from an unheated Southwestern cotton honey (No. HS-38) by the first four steps of the method previously described[1]. It had a specific activity of 224 units/mg (see ref. 1 for definition of units).

All experiments were run at 20° to minimize the rate of mutarotation of the sugars while allowing the oxidation to proceed at a measurable rate. Even then the mutarotation was much more rapid than the oxidation, requiring that corrections be made for it. The mutarotation curves of both α- and β-D-glucose were determined upon the dissolution of 0.04 g/ml of each sugar in 0.2 M sodium phosphate buffer, pH 6.1, and subsequent measurement in 2-dm, jacketed tubes in a Bellingham and Stanley (London) polarimeter, using the sodium-D line as the light source. Equilibrium was reached in about 35 min. Calculation of the mutarotation constant gave $K=0.151$ min^{-1} (where $K = \frac{1}{t_2 - t_1} \ln \frac{\theta_1 - \theta_{eq.}}{\theta_2 - \theta_{eq.}}$), indicating a mutarotation rate in buffer about 10 times faster than that previously found for these sugars in water[2-4]. Using the theoretical formulae of KEILIN AND HARTREE[5], the concentrations of α- and β-D-glucose present at any time during the process of mutarotation were calculated. With these figures it was then possible to correct for the effect of mutarotation on the concentration of each anomer during the oxidation reaction.

The oxidation of α- and β-D-glucose by the honey glucose oxidase was followed manometrically by measurement of the O_2 uptake under standard Warburg procedures. The mainspace of the vessels generally contained enzyme in buffer, with the addition of catalase and ethanol (as recommended by KEILIN AND HARTREE[6]). Substrates were introduced as solids from side-arm sacs. The center wells contained alkali.

An experiment run at the previously determined[1] optimum substrate concentration for equilibrium glucose (1.5 M) required 945 mg of each anomer. This made dissolution difficult but still possible by using finely powdered sugars and extensive mixing. As seen in the plots of the observed data (open circles) of Fig. 1, the oxidation of β-D-glucose proceeds at a rate greater than that of α-D-glucose ($\beta/\alpha = 1.6/1$). This compares favorably with the findings of BEAN AND HASSID[7] for the red alga enzyme ($\beta/\alpha = 1.7/1$). After correction for mutarotation, the difference between the two anomers is considerably greater (Fig. 1, closed circles), the ratio being $\beta/\alpha = 5.4/1$. In a similar experiment at a lower concentration (1 M) the ratio was $\beta/\alpha = 7.6/1$.

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* Mention of company or trade names does not imply endorsement by the Department over others of a similar nature not named.

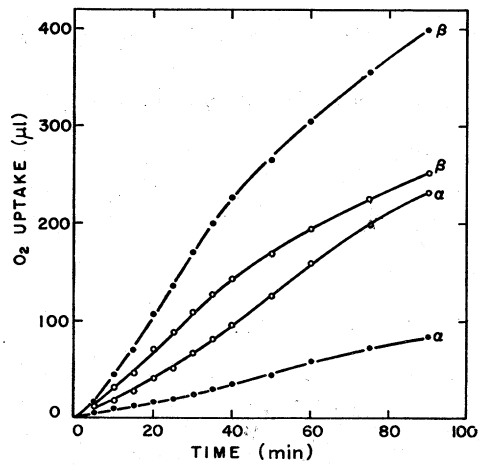

Fig. 1. Manometric measurement of the oxidation of glucose anomers by honey glucose oxidase. Warburg flasks contained 0.2 ml (446 units) glucose oxidase, 0.1 ml (800 Sigma units) catalase, 0.1 ml 95% ethanol and 2.5 ml 0.2 M sodium phosphate, pH 6.1, in the mainspace; 945 mg α-D- or β-D-glucose in side-arm sac; 0.2 ml 10% KOH in well; gassed with O_2. Observed (○—○); corrected for mutarotation (●—●); α = α-D-glucose, β = β-D-glucose.

These results are in contrast with those of KEILIN AND HARTREE[5] for the mold enzyme (β/α = 157/1) after correction for mutarotation.

The change in slope in the observed oxidation of α-D-glucose (Fig. 1) at about the time that mutarotational equilibrium is achieved may be interpreted to mean that initially the oxidation rate is limited by the mutarotation $\alpha \rightarrow \beta$, but once this is completed, the rate increases and parallels the β, showing the presence of principally β-oxidation from then on. Even after correcting for the presence of β in the α sample due to mutarotation, there is still some oxidation of α, indicating that the enzyme can utilize it on a limited scale.

To compare the oxidation rates of β-D-glucose and equilibrium glucose (β = 63.5%, α = 36.5%) at the optimum concentration (1.5 M) for equilibrium glucose required by the enzyme[1], an experiment was run using a previously equilibrated glucose solution and 602 mg of β-D-glucose as substrates. This amount of the β-anomer corresponded to the level present in the equilibrium mixture (63.5% or about 1 M). The resultant oxidation curves were almost identical, when the β-D-glucose was corrected for mutarotation, indicating that the oxidation of 1.5 M equilibrium glucose was equivalent to the oxidation of 1 M β-D-glucose. This agrees with the fact that 1.5 M equilibrium glucose is about 1 M in β-D-glucose.

Using a modification of the apparatus of KEILIN AND HARTREE[5], the possible presence of a mutarotase (D-glucose 1-epimerase, EC 5.1.3.3) in the glucose oxidase preparation was investigated. Results were negative.

An experiment in which the mutarotation was followed during the oxidation reaction showed no effect of the reactants or products on the observed rotation; this was not unexpected from a consideration of the concentrations involved.

We may thus conclude that the glucose oxidase of honey shows a preference for β-D-glucose over α-D-glucose of about 6:1 and that the previously observed[1]

optimum substrate concentration of 1.5 M for equilibrium glucose is in reality 1 M in terms of β-D-glucose.

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